Determination of Residues of Mesurol and Its Sulfoxide and Sulfone in Plant, Animal, and Soil Samples

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A gas–liquid chromatographic method was developed for measuring residues of Mesurol and its sulfoxide and sulfone metabolites in plant, animal, and soil samples. The method involved an initial extraction, a precipitation step to remove waxes and pigments, and a silica gel column cleanup. The sample was then oxidized and/or passed through an alumina–Florisil column. The carbamates were hydrolyzed to the phenols which were silylated for gas–liquid chromatographic detection using a flame photometric detector operated in the sulfur mode. Recovery of the Mesurol compounds from crop, animal, and soil matrices fortified at 0.02, 0.05, 0.1, or 1.0 ppm ranged from 62 to 115%. The lower limit of detection was 0.02 ppm for each compound, and control values were <0.02 ppm.

Mesurol [3,5-dimethyl-4-(methylthio)phenol methylcarbamate], also known as methiocarb, mercaptodimethur, BAY 37344, or Draza, is a carbamate bird repellent, insecticide, and molluscicide. Foliar sprays of Mesurol demonstrate a wide spectrum of effectiveness in deciduous fruit, small fruits, citrus, and ornamentals; baits are effective on various field, fruit, and vegetable crops; seed treatments provide effectiveness on vegetable crops.

Some workers have reported gas chromatographic methods for the detection of Mesurol in residue amounts. Two electron capture detection methods required preparation of either the brominated derivative of Mesurol (Van Middelem et al., 1965) or the ether-boronated derivative following steam distillation of Mesurol (Ernst et al., 1975). These methods had lower limits of detection of 2 and 0.5 ppm, respectively. A microwave emission detector has been used to record sulfur response of Mesurol phenol (Bache and Lisk, 1968). The remaining methods have used a flame photometric detector operated in the sulfur mode for detection of residues of Mesurol, Mesurol sulfoxide, and Mesurol sulfone. The detection of the three carbamates together in plant and animal (Thornton and Dräger, 1973) or soil (Morris and Olson, 1973) required an oxidation of the carbamates to the sulfone, followed by overnight silylation which also hydrolyzed the sulfone to sulfone phenol. Two other methods have separated the three carbamates and three phenols by two cleanup columns (Bowman and Beroza, 1969) or by trifluoroacetylation of the compounds (Greenhalgh et al., 1976) before flame photometric detection in the sulfur mode of the individual compounds.

The following procedure for crops, animal samples (fish, poultry, and eggs), and soil incorporated many of the steps from these previous methods but gave the option of analyzing Mesurol compounds together or separately. An overnight hydrolysis–silylation, used in most previous methods, has been omitted and replaced by a quicker 30-min basic hydrolysis and silylation.

EXPERIMENTAL SECTION

Apparatus. A Hewlett-Packard 5750 gas–liquid chromatograph equipped with a Melpar flame photometric detector operated in the sulfur mode (394-nm filter) with a solvent vent valve was used. A borosilicate glass column (0.7 m × 2 mm i.d.), packed with 5% OV-225 on 60–80-mesh Gas-Chrom Q, solution coated (Applied Science Laboratories, Inc., 1967), was conditioned by heating at 250 °C for 1.5 h with no gas flow and then heating with gas flow at 200 °C for 1 h. Operating parameters were as follows: helium carrier gas, 50 mL/min; other gases set to give maximum response and minimum noise as per manufacturer's recommendation; column oven temperature, Mesurol 125 °C, Mesurol sulfoxide and Mesurol sulfone 180 °C; detector temperature, 180 °C; injection port temperature, 180 °C. A Hobart food chopper was used to process samples, and a Waring Blendor (explosion proof) was used to extract samples (except soil). A centrifuge equipped with head and carriers for 125-mL separatory funnels was needed. A rotary vacuum evaporator was needed to concentrate samples. Water baths at 30 and 60 °C were required.

Reagents. All solvents were pesticide grade. Alumina, acid washed (Merck & Co., Inc., Rahway, NJ), was heated overnight at 110 °C and deactivated by mixing 10 mL of distilled water with 90 g of alumina. The pH 6 buffer solution was made by dissolving 300 g of dibasic sodium phosphate and 25 g of citric acid in distilled water and diluting the mixture to 1000 mL. Florisil (60–100 mesh) (Fisher Scientific Co., Fair Lawn, NJ) was heated overnight at 110 °C and deactivated by mixing 2.5 mL of distilled water with 97.5 g of Florisil. Glass beads were P series, 100–400 mesh (Potter Industries, Carlstadt, NJ). Hyflo Super-Cel (Johns-Manville Products Corp., Lompoc, CA) was used as a filter aid. The keeper solution was 1% mineral oil in toluene. Magnesium sulfate was made 20% (w/v) in distilled water. Potassium permanganate was made 0.1 M in distilled water. Precipitating solution was prepared by dissolving 1.25 g of ammonium chloride and 25 mL of phosphoric acid in distilled water and diluting to 1000 mL. Regisil [*N*,*O*-bis(trimethylsilyl)trifluoroacetamide, BSTFA] was obtained from Regis Chemical Co., Morton Grove, IL. Silica gel was used as received (J. T. Baker Chemical Co., No. 3405). Sodium hydroxide was prepared 10% (w/v) in distilled water. Sodium sulfate, anhydrous, was used in both granular and powder forms. Sulfuric acid was diluted to 5% (v/v) with distilled water.

Standards. A standard solution of Mesurol, Mesurol sulfoxide [3,5-dimethyl-4-(methylsulfinyl)phenol methylcarbamate], or Mesurol sulfone [3,5-dimethyl-4-(methylsulfonyl)phenol methylcarbamate] contained 1 mg of Mesurol compound/mL of methanol. A working solution contained 5 or 10 μg of Mesurol compound/mL. Solutions were kept frozen for an indefinite period of time.

Sample Preparation. Wet crops and animal tissues were ground in a food chopper in the presence of dry ice, and the sample was placed in frozen storage overnight to allow the dry ice to sublime. Dry crops or soil were pul-

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verized with a mortar and pestle or food chopper.

Extraction of Crops. A 100-g portion of the sample was weighed into a blender jar. For bulky crops, 350 mL of acetonitrile was added; for dry crops, 50 mL of distilled water and 250 mL of acetonitrile were added; for all other crops, 250 mL of acetonitrile was added. The sample was blended at moderate speed for 3 min and filtered through a 9-cm Whatman No. 541 filter paper covered with a 4-g layer of Hyflo Super-Cel in a Büchner funnel. The filter cake was returned to the blender jar, and the blending was repeated, omitting the water for dry crops. The brei was filtered as before, but no additional Hyflo Super-Cel was used. The blender jar was rinsed with 50 mL of acetonitrile, and this rinse was used to wash the filter cake. The filtrate was transferred to a 1000-mL separatory funnel, 250 mL of hexane was added, and the separatory funnel was shaken for 30 s. The phases were allowed to separate, and the acetonitrile was drained into a 1000-mL flask and evaporated just to dryness by using a rotary evaporator with a 30 °C water bath. The sample was reserved for Precipitation.

Extraction of Animal Tissues. (This was for poultry and fish.) A 50-g portion of the sample was weighed into a blender jar and blended in 200 mL of acetonitrile at moderate speed for 1 min. Fifty grams of powdered sodium sulfate was added, and blending of the sample continued for 2 min more. The sample was filtered through a 9-cm Whatman No. 541 filter paper covered with a 4-g layer of Hyflo Super-Cel in a Büchner funnel. The filtrate was transferred to a 500-mL separatory funnel. The filter cake was returned to the blender jar and blended with 200 mL of hexane for 2 min. (For fat and skin samples, the order of blending with acetonitrile and hexane was reversed; i.e., the sample was blended first with hexane.) The sample was filtered as before, but no additional Hyflo Super-Cel was used. The filtrate was combined with the acetonitrile in the separatory funnel. The blender jar was rinsed with 100 mL of hexane, and this rinse was used to wash the filter cake. The wash was combined with the solvents in the separatory funnel, the separatory funnel was shaken for 30 s, and the phases were allowed to separate. The lower layer was drained into a second 500-mL separatory funnel containing 250 mL of fresh hexane, the second separatory funnel was shaken for 30 s, and the phases were allowed to separate. The lower layer was drained into a 500-mL flask. The two-step extraction was repeated with 50 mL of fresh acetonitrile, and the extracts were combined. The combined acetonitrile fractions were evaporated just to dryness at 30 °C. For skin and fat samples, the sample was reserved for Precipitation. For other samples, 50 mL of benzene was added to the flask and evaporated to dryness at 30 °C to remove all acetonitrile which would interfere with oxidation. The sample was reserved for Oxidation.

Extraction of Eggs. An egg was weighed into a blender jar, blended with 200 mL of acetone at moderate speed for 3 min, and filtered through a 9-cm Whatman No. 541 filter paper covered with a 4-g layer of Hyflo Super-Cel in a Büchner funnel. The blender jar was rinsed with 50 mL of fresh acetone, and this rinse was used to wash the filter cake. The filtrate was transferred to a 1000-mL separatory funnel. The blender jar was rinsed with 300 mL of chloroform, and this rinse was used to wash the filter cake. The combined chloroform and acetone in the separatory funnel was shaken for 30 s, and the phases were allowed to separate. The lower layer was drained through a 32-cm Whatman No. 2V fluted filter paper into a 1000-mL flask and evaporated just to dryness at 30 °C. The residue in the flask was transferred to a 125-mL separatory funnel with 50 mL of hexane. The flask was rinsed with 25 mL of acetonitrile, and the acetonitrile was combined with the hexane rinse. The separatory funnel was shaken for 30 s, and the phases were allowed to separate. The acetonitrile was drained into a second 125-mL separatory funnel containing 25 mL of fresh hexane, the second separatory funnel was shaken for 30 s, the phases were allowed to separate, and the acetonitrile was drained into a 125-mL flask. The two-step extraction was repeated with 20 mL of fresh acetonitrile. The combined acetonitrile fractions were evaporated just to dryness at 30 °C. Fifteen milliliters of benzene was added to the flask and evaporated to dryness to remove all acetonitrile which would interfere with oxidation. The sample was reserved for Oxidation.

Extraction of Soil. A 100-g dried soil sample was weighed into a Soxhlet thimble and extracted for 16 h with 400 mL of chloroform–methanol (7:3) and boiling chips. The extract was evaporated to 1–2 mL at 30 °C. The sample was reserved for Precipitation.

Precipitation. (This was for crops, poultry skin and fat, and soil.) The residue in the flask was dissolved in 40 mL of acetone, 50 mL of precipitating solution was added, and the suspension was mixed well. The flask was allowed to stand at room temperature for 30 min with occasional swirling. The solution was filtered through a 4.25-cm Whatman No. 2 filter paper covered with 1-g layer of Hyflo Super-Cel in a Büchner funnel. The sides of the flask were rinsed with 20 mL of acetone and 25 mL of precipitating solution, and the filter cake was washed with the mixture. This washing step was repeated with 20 mL of fresh acetone and 25 mL of fresh precipitating solution. The filtrate was transferred to a 250-mL separatory funnel. The flask was rinsed with 50 mL of chloroform, and the rinse was added to the separatory funnel. After the separatory funnel was shaken for 30 s and the phases were allowed to separate, the lower layer was drained into a 500-mL flask. The extraction was repeated with two additional 50-mL portions of fresh chloroform. The combined chloroform was evaporated just to dryness at 30 °C. For crops, the sample was reserved for Silica Gel Column Cleanup. For skin, fat, and soil, the sample was reserved for Oxidation.

Silica Gel Column Cleanup. (This cleanup was necessary for some crops, including bean vine, orange peel, lettuce, broccoli, brussels sprout, and cauliflower head and leaf. For other crops, the sample was reserved for Oxidation). A chromatographic column was prepared by adding successively a plug of glass wool, 2 g of glass beads, acetone to fill the column, 5 g of silica gel, and 2 g of granular sodium sulfate. The acetone was drained to the top of the sodium sulfate and discarded. A 125-mL flask was placed under the column. The residue in the flask (from the Precipitation step) was rinsed into the column with three 5-mL portions of acetone, each wash being transferred to the column after the previous wash had passed into the sodium sulfate. The column was eluted with an additional 35 mL of acetone. The acetone was evaporated just to dryness at 30 °C. The sample was reserved for Oxidation.

Oxidation. (For analysis of Mesurol, Mesurol sulfoxide, and Mesurol sulfone separately, the oxidation was omitted. The sample was reserved for Alumina–Florisil Column Cleanup.) A corresponding standard was started at this point. The residue in the flask was dissolved in 2 mL of acetone. To sample and standards, 5 mL of magnesium sulfate solution and 30 mL of potassium permanganate solution were added, the flask was swirled to mix the so-

lution, and the solution was allowed to stand at room temperature for 10–15 min with occasional swirling. (The solution was not allowed to stand longer than 15 min.) The solution was transferred to a 125-mL separatory funnel, the flask was rinsed with 25 mL of chloroform, and the chloroform was added to the separatory funnel. The separatory funnel was shaken for 30 s, the phases were allowed to separate, the solution was centrifuged if necessary, and the lower layer was drained through a plug of glass wool in a funnel into a 250-mL flask. The extraction was repeated twice more with fresh 25-mL portions of chloroform. Keeper solution (8 drops) was added to the flask, and the combined chloroform extracts were evaporated just to dryness at 30 °C. For animal tissues, the sample was reserved for Alumina–Florisil Column Cleanup. For other samples, the sample was reserved for Hydrolysis and Silylation.

Alumina–Florisil Column Cleanup. (This cleanup was for animal tissues, eggs, and any crop or soil not oxidized.) A chromatographic column was prepared by adding successively a plug of glass wool, 2 g of glass beads, ethyl acetate to fill the column, 10 g of Florisil, 5 g of alumina, and 2 g of granular sodium sulfate. The ethyl acetate was drained to the top of the sodium sulfate and discarded. The residue in the flask was rinsed into the column with three 5-mL portions of ethyl acetate, each wash being added to the column after the previous wash had passed into the sodium sulfate. The column was eluted with an additional 185 mL of ethyl acetate. The ethyl acetate was evaporated just to dryness at 30 °C. The sample was reserved for Hydrolysis and Silylation.

Hydrolysis and Silylation. (For analysis of the carbamates separately, a corresponding Mesurol standard was begun here.) The residue in the flask was dissolved in 1–2 mL of acetone, and 10 mL of sodium hydroxide solution and 8 drops of keeper solution were added to the flask. The flask was placed in a 60 °C water bath for 30 min, the flask being swirled occasionally. The flask was cooled to room temperature, and the solution was transferred to a 125-mL separatory funnel. The flask was rinsed with 10 mL of chloroform, and the rinse was added to the separatory funnel. The separatory funnel was shaken for 30 s, the phases were allowed to separate, the separatory funnel was centrifuged if necessary, and the chloroform was discarded. This basic wash was repeated with two additional 10-mL portions of chloroform. (It was not necessary to do the chloroform basic washes with the standards.) The solution in the separatory funnel was acidified with 15 mL of sulfuric acid solution and then partitioned with three 25-mL portions of chloroform, and the chloroform was passed into a 125-mL flask through ~10 g of powdered sodium sulfate retained in a funnel by a plug of glass wool. The sodium sulfate was rinsed with an additional 15 mL of chloroform. Keeper solution (8 drops) was added to the flask, and the combined chloroform was evaporated just to dryness at 30 °C. The residue in the flask was transferred with acetone to a 5-mL graduated tube, and the acetone was evaporated to 0.9 mL or less with a stream of nitrogen. Regisil (0.1 mL) was added, the solution mixed well, and the volume adjusted to 1.0 mL acetone just prior to injection.

Gas Chromatographic Analysis. Derivatized extract (20 μL) was injected into the gas chromatograph, and the solvent was vented for ~0.5 min. The derivatized Mesurol, Mesurol sulfoxide, or Mesurol sulfone peak was identified by its retention time, and the response (peak height) was measured. At the operating conditions employed, the retention time for silylated Mesurol phenol or silylated Mesurol sulfoxide phenol was 3 min at 125 and 180 °C, respectively, and for silylated Mesurol sulfone phenol was 4 min at 180 °C.

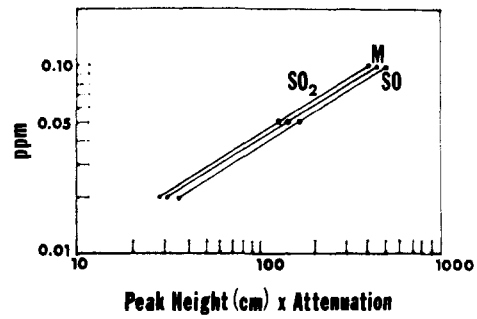

Figure 1. Example of log–log plot for Mesurol (M), Mesurol sulfoxide (SO), and Mesurol sulfone (SO_2).

Preparation of Standard Curve. A series of standard solutions that contained from 50 to 200 ng of Mesurol, Mesurol sulfoxide, or Mesurol sulfone that had been converted to the corresponding phenol and silylated with Regisil were injected. The peak heights were measured, and the height for each peak was multiplied by the electrometer attenuation. Ppm equivalence of each standard injected was determined by using the formula

ppm of standard =
[(ng of standard injected)/[sample wt (g)]] ×
[[final sample volume (mL)]/(μL of sample injected)]

A standard curve was plotted from the values for each compound by using log–log graph paper (Figure 1). The log–log plot was necessary to obtain a straight line plot because of the logarithmic response of the flame photometric detector in the sulfur mode (Brody and Chaney, 1966). Values for samples analyzed were then read from the standard curve directly in ppm. Any samples that contained residues high enough that the amount injected exceeded 200 ng were diluted so that response fell on the plotted curve. For those samples, the residue calculation was

ppm in sample =
(ppm from standard curve) × (dilution factor)

A new standard curve was prepared each day.

RESULTS AND DISCUSSION

This method was designed to measure residues of Mesurol, Mesurol sulfoxide, and Mesurol sulfone in crops of all types, animal tissues (poultry, eggs, and fish), and soils, with elimination of the corresponding Mesurol phenol compounds. The three Mesurol carbamate compounds could be measured together as one peak or individually.

If the three Mesurol carbamate compounds were to be determined together, the oxidation step converted the Mesurol and Mesurol sulfoxide to Mesurol sulfone with subsequent gas chromatographic analysis of only one peak (Mesurol sulfone phenol) for convenience and enhanced sensitivity. Room temperature oxidation to the sulfone was quantitative using 0.1 M potassium permanganate for 15 min. Those oxidation conditions did not affect the carbamate portion of the molecule. Oxidation also converted most tissue extractives and pigments to water-soluble forms, making them easy to remove (Tietz and Frehse, 1960). The corresponding Mesurol phenols were also eliminated.

If the Mesurol carbamate compounds were to be determined individually, the oxidation step was omitted. An alumina–Florisil column was included in the method to separate the tissue extractives, pigments, and the corre-

Table I. Recovery of Mesurol and Metabolites from Crops, Animal Tissues, Eggs, and Soil

sample	ppm added	% recovery Mesurol	sulfoxide	sulfone
crops[a]				
artichoke	0.1	81	73	81
	0.05	84	70	82
bean, dry	0.1	100	81	97
	0.05	95	86	92
bean and pod, mixed	0.1	99	101	103
	0.05	100	100	100
bean vine	0.1	90	102	100
	0.05	104	85	91
broccoli	0.1	94	96	107
	0.05	93	101	102
brussels sprout	0.1	100	98	101
	0.05	99	102	102
cabbage	0.1	93	112	104
	0.05	85	110	100
cauliflower head	0.1	106	100	103
	0.05	101	92	95
cauliflower leaf	0.1	73	75	80
	0.05	76	71	75
cherries[b]	0.1	62	81	86
	0.05	72	65	66
lettuce	0.1	78	89	99
	0.05	98	94	104
orange peel	0.1	92	102	103
	0.05	100	95	92
orange pulp	0.1	101	99	104
	0.05	103	98	103
peaches[b]	0.1	74	83	89
	0.05	64	98	85
tomato	0.1	99	100	101
	0.05	104	100	103
fish[b]				
edible	1.0	80	90	80
offal	1.0	80	90	77
poultry[a]				
muscle	0.1	75	88	88
	0.05	93	80	90
giblet	0.1	100	100	100
	0.05	98	98	96
skin	0.1	74	86	85
	0.05	93	90	96
fat	0.1	101	105	115
	0.05	103	106	101
egg	0.1	80	79	73
	0.05	79	84	78
	0.02	88	88	115
soil[b]				
sand	0.05	84	96	96
sandy loam	0.05	83	94	96
silt loam	0.05	74	92	98
silt loam with high organic matter	0.05	72	95	100

[a] Mesurol, Mesurol sulfoxide, and Mesurol sulfone were added to separate samples at the blending step. [b] Mesurol, Mesurol sulfoxide, and Mesurol sulfone were added together at the blending step.

sponding Mesurol phenols from the carbamates. (In animal tissues, the column was needed for cleanup whether the oxidation step was used or not.) Acid-washed alumina was used to obtain adequate recovery.

The crop extraction procedure using acetonitrile–hexane (Thornton and Dräger, 1973) was adopted as the general extraction method. This procedure removed the nonpolar oils and lipids, thus creating fewer difficulties from coextractives in the subsequent steps.

The carbamate compounds, after being subjected to oxidation and/or alumina–Florisil column cleanup, were hydrolyzed to their corresponding phenols. This hydrolysis was needed because the carbamates undergo partial decomposition during the gas chromatography step. Also, the retention times of the intact carbamates were such that interferences from other sulfur-containing insecticides were introduced. Therefore, the 30-min basic hydrolysis (Bowman and Beroza, 1969) was used here.

Better gas chromatographic results were obtained with the silylated phenols than with the free phenols or other derivatives; therefore, the phenols produced by the basic hydrolysis were reacted with Regisil just prior to injection.

For enhancement of response and more stable operation, the flame photometric detector was modified (1) by removal of the flame ionization collector ring, (2) by installation of a valve to vent solvent so the detector was kept cleaner, and (3) by reversal of the hydrogen and air/oxygen supply lines so the flame was not extinguished when the sample was injected without the solvent being vented.

Recovery experiments were caried out with various crops, eggs, poultry and fish tissues, and soils. Known amounts of the Mesurol compounds were added to the sample at the extraction step. Table I gives the sample types, ppm of Mesurol compounds added, and the recovery obtained. Controls showed no interferences for any Mesurol compound. The lower limit of detection of the method was 0.02 ppm.

Chloroform was used throughout the method. Because of possible hazards involved with the use of chloroform, methylene chloride has been tried as a replacement. Identical results have been obtained with methylene chloride as compared to chloroform in the samples tested.

The method outlined in this paper measured residues of Mesurol, Mesurol sulfoxide, and Mesurol sulfone either together or individually. The method eliminated interferences with coextractives and Mesurol phenol compounds and had good sensitivity.

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